Molecular characterisation of NDM-1-bearing K. pneumoniae isolates from Croatia
A. Mazzariol*, E. Kocsis, M. Gužvinec, I. Butic, S. Kresic, A. Tambic, G. Cornaglia (Verona, IT; Zagreb, Bjelovar, HR)

Objective: Molecular genetic characterization of 4 blaNDM-1 positive K. pneumoniae strains isolated from Croatia. Methods: Four carbapenem-resistant Klebsiella pneumoniae were isolated from clinical samples from four different hospitals. Three of them are situated in Zagreb and one in Bjelovar. Species identification was carried out by API. MICs were performed by microdilution method and interpreted by the EUCAST criteria. Investigation of genes encoding for beta-lactamases (ESBL, MBL, OXA-type and plasmidic AmpC), plasmid-mediated quinolone resistance determinants (qnrA, qnrB, qnrS, qnrC, qnrD, qepA, aac(6’)-Ib-cr variant) and 16S rRNA methylase (armA, rmtB, rmtC) was performed by PCR. PCR-based replicon typing provided the plasmid scaffold of the strains. Multilocus sequence typing (MLST) was performed. Conjugation experiment was also carried out. Results: All the strains were blaNDM-1 positive. MLST yielded sequence type (ST) 15 (3 strains) and ST16 (one strain). Three ST15 strains produced, also TEM-1, SHV-12, CTX-M-15, OXA-1- group beta-lactamases and harboured qnrB1 gene. All three possessed a common plasmid that, surprisingly, was IncR and not A/C as described in most previous reports. Beside IncR plasmid, one strain harboured colE and the third strain carried both L/M and colE plasmids. The K. pneumoniae strain belonging to ST16 possessed A/C and colE plasmids and harboured CIT-type acquired AmpC gene together with blaOXA-1, blaCTX-M-15, blaSHV-1, blaTEM-1. The transconjugants obtained from IncR type strain were positive for blaNDM-1 and IncR genes by PCR. Conclusion: This study revealed diverse genetic features among several NDM-1 positive K. pneumoniae strains isolated in four Croatian hospitals. The results confirmed the presence of multiple beta-lactamase genes other than NDM-1 as well the presence of determinants of resistance to other antibiotic classes, as typical for these strains. The plasmid profiles revealed the presence of either one or two plasmids, with different patterns among the strains. The finding of IncR plasmids, so far not reported, is of particular relevance.